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COMMENTARY

Use of the NASA Space Radiation Laboratory at Brookhaven National Laboratory to Conduct Charged Particle Radiobiology Studies Relevant to Ion Therapy

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Although clinical studies with carbon ions have been conducted successfully in Japan and Europe, the limited radiobiological information about charged particles that are heavier than protons remains a significant impediment to exploiting the full potential of particle therapy. There is growing interest in the U.S. to build a cancer treatment facility that utilizes charged particles heavier than protons. Therefore, it is essential that additional radiobiological knowledge be obtained using state-of-the-art technologies and biological models and end points relevant to clinical outcome. Currently, most such ion radiotherapy-related research is being conducted outside the U.S. This article addresses the substantial contributions to that research that are possible at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL), which is the only facility in the U.S. at this time where heavy-ion radiobiology research with the ion species and energies of interest for therapy can be done. Here, we briefly discuss the relevant facilities at NSRL and how selected charged particle biology research gaps could be addressed using those facilities.

INTRODUCTION

Charged particles have significant potential advantages in cancer therapy compared to photons because they deposit relatively little absorbed dose at the surface of a patient's body (as well as little dose to normal tissues located beyond the depth of a tumor), while depositing most of their energy just before they come to rest in a tumor target at the Bragg peak. In addition to this physical dose distribution advantage, ions heavier than protons have increased effectiveness for cell killing and damage, depending on the density of the ionizations in the particle track, known as linear energy transfer (LET), and on the level of hypoxia in the tumors irradiated. Although clinical studies with charged particles heavier than protons have been conducted successfully in Japan and Germany, the limited radiobiological information about charged particles remains a significant impediment to exploiting the full potential of particle therapy. At the 2013 Workshop on Ion Beam Therapy sponsored by the U.S. Department of Energy (DOE) and the National Cancer Institute (NCI) (*1*), it was concluded that for future optimization of ion therapy it is essential to obtain additional radiobiological knowledge using state-of-the-art technologies and biological models and end points relevant to clinical outcome. Subsequently, the NCI issued the funding opportunity announcement, ''Planning for a National Center for Particle Beam Radiation Therapy Research (P20)'', which resulted in the funding of two centers: the Texas Center for Advanced Radiation Therapy (TCART; UT Southwestern Medical Center, Dallas, TX) and the North American Particle Therapy Alliance (NAPTA; San Francisco, CA).

Currently, most radiotherapy-related heavier-than-protons biology research is being completed outside the U.S., although a substantial contribution to that research could be achieved through the use of the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL), currently the only facility in the U.S. where heavy-ion radiobiology research with the ion species and energies of interest for therapy can be done. Here, we address the radiobiological research needed to aid development of ion beam therapy and the potential utilization of NSRL facilities to address those needs. Relevant facilities at NSRL are described briefly followed by a discussion of charged

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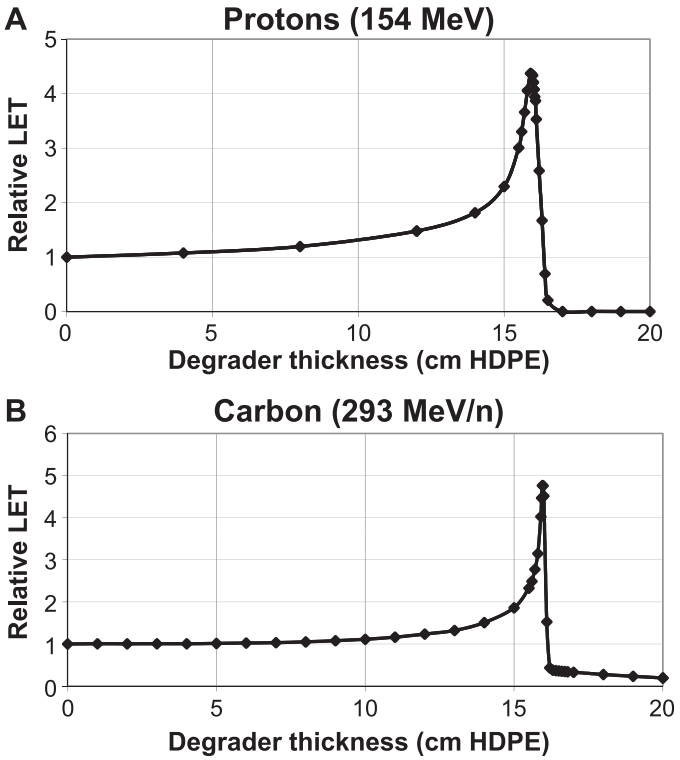

FIG. 1. Bragg curves obtained at NSRL for 154 MeV protons (panel A) and 293 MeV/n carbon ions (panel B) measured in high-density polyethylene (HPDW, $\rho = 0.97$ g/cm^3). LET on entrance of protons is 0.45 keV/μm and of carbon ions is 24.33 keV/μm. In both cases the particle range is 16 cm. [From the NSRL User Guide (*15*), used with permission].

particle biology research gaps with an emphasis on how NSRL facilities could be used to address them.

NSRL FACILITIES

NSRL was designed and built through a NASA-funded, DOE-managed collaboration to provide charged particle beams for radiobiology research in support of NASA's humans-in-space initiative. The facilities have been previously described elsewhere (*2, 3*), and the salient features important for their use in ion beam therapy research are summarized here. NSRL consists of a particle beam transport system emanating from the booster synchrotron to a dedicated experimental area and support building. The facility can provide a wide range of ions from protons to uranium, with energies from 50 to 2,500 MeV/n. For radiation therapy-related research, the species of interest, i.e., protons to neon with energies up to about 400 MeV/n, are readily available. Examples of Bragg curves for protons and carbon ions obtained at NSRL are shown in Fig. 1. Doses and dose rates as low as 100 particles/cm^2 and up to about 4 Gy/min, depending on ion species and field size, can be provided. Beam is delivered into a 400 sq. ft. target hall connected to a 4,560 sq. ft. support building, which includes five laboratories for biological and materials experiments, as well as specimen handling, dosimetry and control rooms.

Individuals wishing to use NSRL must submit a beam time request, which is reviewed by BNL's Scientific Advisory Committee for Radiation Research (SACRR) for feasibility and appropriateness of proposed studies. SACRR makes recommendations on the beam time applications to BNL's Associate Laboratory Director for Nuclear and Particle Physics for assignment of beam time based on BNL established policy (*3*). Projects must have some form of documented scientific peer review to be granted beam time. For projects not funded by NASA there is an hourly charge. Scheduling of ions and beam time is arranged by personnel in BNL's Collider-Accelerator Department and NSRL Support Group. For radiobiological research, NSRL typically operates 5 days a week at 8 science hours per day. Unused beam time is available for other users, provided there is no interference with the NASA program. All users are required to undergo BNL training as appropriate for their proposed experiments, e.g., radiation safety, biological hazards, and all projects are reviewed for compliance with BNL safety standards.

NSRL support group staffers provide support for the NSRL facility and for users during their runs at the NSRL. NSRL is fully equipped for animal care during irradiations and for cell work, e.g., including cell culture hoods, incubators, microscopes, water baths, etc. BNL personnel conduct the irradiation beam set-up, dosimetry and all operations. Before and after irradiations at NSRL, the Long-Term Support Facility (LTSF) and the Brookhaven Laboratory Animal Facility (BLAF), located at the Biology Department, provide the infrastructure support for life sciences research. Laboratories and offices within the LTSF house facilities for cell and tissue culture, molecular biology, flow cytometry and rodent studies. NSRL support group staff provides primary support and operation of the LTSF, assisting users with all operational needs. At both NSRL and in LTSF and BLAF, if specialized equipment is required, staff members work closely with users to satisfy needs as much as possible.

CHARGED PARTICLE BIOLOGY RESEARCH NEEDS

Biologically Effective Doses

High-LET ions are more effective at killing cells and causing other cell and tissue damage than low-LET ion species, usually expressed in terms of relative biological effectiveness [RBE; ratio of doses for any given biological effect induced by a low-LET reference radiation, (historically 250 kVp X rays but now usually ^{60}Co gamma rays), and the high-LET radiation of interest]. It is well established that RBE increases with LET to a maximum at 50–200 keV/μm, depending on the ion species, then decreases at very high LET (*4*). However, the absolute RBE values depend on numerous factors including the specific charged particle and

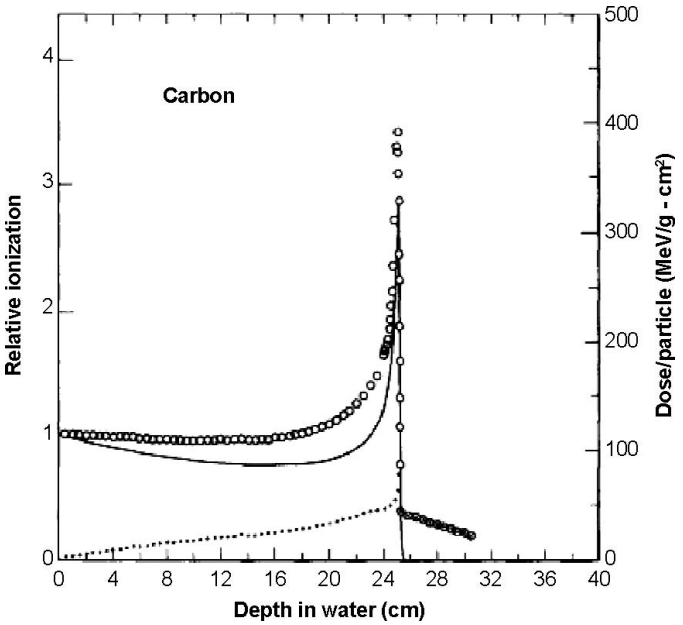

FIG. 2. Calculated (solid line) and measured Bragg peaks for 400 MeV/n carbon ions. The ''extra'' dose due to the fragmentation tail is indicated. [From Blakely *et al*. (*16*), used with permission.]

its energy, cell or tissue type, end point and the dose and dose rate. Much of the clinical work with protons and carbon ions has been based on the use of RBE values derived under relatively limited and specific experimental circumstances. Such ''generic'' RBE values may not be the most appropriate for a range of tumor types or for all normal tissues. For example, early responding normal tissues tend to have lower RBE values than late responding tissues and various tumor types can have quite different RBE values depending, at least in part, on photon α/β ratios. Furthermore, the physical characteristics of particle species and the energy deposition patterns of ion beams change as particles traverse and finally stop in patients. Radiation safety and clinical effectiveness require experimental confirmation of RBE values in both normal tissues and tumors and increased understanding of the complex interactions of different ionization densities along the beam path. Although clinical trials comparing photons, protons and heavier ions can, and are, being planned based on matching the physical dose conformality as much as possible, the inaccuracy in RBE knowledge remains the main source of uncertainty in dose prescription in carbon ion therapy (*5*). In addition, the much more limited knowledge of RBE values for other ions makes it extremely difficult to predict biologically effective doses and subsequent outcomes in patients.

With the exception of the early charged particle clinical studies at Berkeley National Laboratory (*6*), most clinical studies reported to date have been with protons and carbon ions (*5, 7, 8*). There is a significant volume of radiation biology literature of relevance to ion therapy for certain ions [summarized in ref. (*6*)], but some of the studies are variable in completeness and, in particular, there is a lack of data on ions between helium and carbon, and for oxygen, ions that may have substantial clinical benefits in terms of dose distributions and biological advantages (*5*). Ideally the initial clinical facility/facilities built in the U.S. would have capabilities to accelerate all ions from protons to oxygen or neon, but given the realities of construction costs, it may be necessary to limit those ion choices. Thus, it is imperative to have experimental radiobiological studies with other ion species, in comparison with protons and carbon, to facilitate decisions on which particles should be made available in a clinical facility.

For these reasons, there is clearly a need for determination of biological responses using clinically relevant doses, dose rates and fractionation schedules to develop appropriate RBE values using a rationally selected set of tumor types and normal tissues, with ion species from helium to oxygen or neon of varying energies, and thus a range of LETs. The studies should include assessment of the biological responses across the entire Bragg curve, including the potential importance of the high-LET dose effects in normal tissues due to ion beam fragmentation in the distal tail of the Bragg peak (Fig. 2). The most relevant end point to be used *in vitro* is cell inactivation, the ''gold standard'' for assessment of response relevant for tumor cure (*4*). Other useful information includes DNA damage response, gene expression, molecular and biochemical changes, mode of cell death, intra- and intercellular signaling and neoplastic transformation. In addition to *in vitro* studies, *in vivo* assessments of tumor and normal tissue responses are critical for obtaining the requisite information. The facilities currently available at NSRL are appropriate for conducting many of these needed studies. NSRL can provide beams of the range of ions and energies desired, and physics personnel are available to assist with experimental setup and dosimetry. Facilities are available for conducting cell studies, and have been used successfully by NASA-funded investigators for a number of years [e.g., see radiation studies in ref. (*9*)], albeit with emphasis on lower doses than those needed for radiation therapy relevant studies. There has been minimal *in vivo* tumor radiobiology research done to date at NSRL. However, relatively straightforward investigations of various ions could be performed now using human tumor xenografts grown subcutaneously on mouse hind limb, for example, with some physics effort required to enable delivery of a spread-out Bragg peak (SOBP) to the tumor and appropriate dosimetry. Dosimetry would be facilitated by the development of collaborations with experts who use Monte Carlo based treatment planning systems for charged particles [e.g., TOPAS (*10*)].

It also has been suggested that there could be clinical advantages to combining two or more ion beam treatments, either simultaneously (or in very rapid succession) or on separate days, for an individual patient. For example, one could envision use of a lighter particle such as protons or helium to irradiate a large tumor volume, and then follow

with a boost dose to a radioresistant (e.g., hypoxic) portion of a tumor with oxygen or neon. However, there is little radiobiological information on the efficacy or potential detrimental side effects of such ion combination treatments. NSRL has the capabilities to produce multiple ion beams and switch between them readily, so that the potential for ion combination treatments could be assessed in appropriate models.

Dose Fractionation

Clinical studies with both photons and charged particles have been performed to explore the safety and efficacy of reducing the number of dose fractions, even down to a single high-dose treatment for selected tumor types, and the favorable tissue depth–dose distributions of charged particles could be used effectively in hypofractionated therapy (*5, 11*). However, the radiobiological effects of single high-dose fractions, e.g., 25 Gy, may be substantially different from those of the 1.8–2.0 Gy dose fractions used in conventional photon radiotherapy (*12*). Among other possible effects, with single high doses one may see greater damage to vasculature and tumor stroma or greater stimulation of immune responses. Such possible differentials could be further enhanced with high-LET radiations. The radiobiological effects of high- and low-LET radiations at high- versus low-dose fraction intensities should be carefully studied in multiple tumor types and in both early and late responding normal tissues, where clinical experience with neutrons has shown that there are significant differences. Varying intensities of ion beams are available at NSRL for all the particles of potential clinical interest, so *in vitro* cellular studies at high versus low single doses could be conducted readily. As noted above, *in vivo* tumor studies with subcutaneous xenograft models would require a relatively modest initial physics and dosimetry development effort. However, for these studies, use of orthotopic tumors might be preferred and parallel studies of normal tissues are vital; to do that at NSRL would require a significantly greater effort in physics and dosimetry development to achieve dose distributions of accuracy similar to those used clinically or that are now available with X-ray systems for small animal studies (*13*). In particular, ''on-board'' imaging, such as X ray, ultrasound, CT and/or luminescence, would be needed for most orthotopic models, and such capabilities do not currently exist at NSRL.

Hypoxia

Tumors can outgrow their oxygen supply and develop regions of hypoxia that are relatively radioresistant to conventional, low-LET radiation therapy. Radiobiological studies have shown that the dependence of cell killing on oxygen decreases as the mass of the irradiating ion species increases, which has led to the long-held belief that heavy ions have an additional clinical advantage of increasing effects on hypoxic tumors (*4*). However, the dependence of this effect on ion species, energy, dose and dose rate are still not clear. Furthermore, tumor reoxygenation during the prolonged course of a typical clinical photon treatment regimen may, in some tumor types, increase photon treatment efficacy. The roles of hypoxia and reoxygenation in ion beam therapy remain important topics where there is a need for radiobiological research. Facilities at NSRL could be used currently for *in vitro* studies of the magnitude of the oxygen effect as a function of LET using systems such as those that have been used with photons for many years (*14*). Assuming the developments in physics and dosimetry at NSRL mentioned in previous sections, *in vivo* studies of hypoxia-related questions could be initiated readily at NSRL.

Altered Dose Rate

The delivery of ion beam therapy via passively scattered beams is being replaced by active beam scanning. Active scanning produces beams with significantly higher instantaneous dose rates (hundreds of Gy/min within a pulse of a few milliseconds) than experienced with traditional dose delivery. This raises the possibility of altered biological responses because of changes in the nano-scale distribution of free radical species and the potential for cellular oxygen depletion. Therefore, research is needed to define the potential impact of beam spill structure and repetition rate on biological and clinical outcomes. Spot scanning at NSRL has not been implemented and would require significant effort and funding to develop. An alternative approach that is worth considering is rastering the animal through a stationary, mm-sized beam, rather than scanning the beam, to achieve a scanned-beam-like dose distribution.

EXAMPLES OF SPECIFIC RADIOBIOLOGY PROPOSALS

Based on the discussion above, this section provides examples of specific radiation biology studies that could be undertaken immediately (pending available funding) using existing facilities at NSRL. The list is followed by experimental sets that could be undertaken with modest development of capabilities at NSRL. These lists are meant to represent potential starting points for much needed biological studies, and it is expected that the user community would have other models or ideas that are important and novel. A description of and cost estimates for the facility modifications that would be needed to accommodate future programs can be developed as needed.

Recommended Heavy-Ion Radiobiological Investigations that Could Be Undertaken Now at NSRL

1. *In vitro* determination of RBE values for clonogenic cell survival and DNA damage induction and repair kinetics in a range of human tumor and normal cell types exposed to various charged particles of interest.

a. Determine full Bragg curves for cell inactivation at a single dose (e.g., 2 Gy) as a function of depth in tissue-equivalent material, e.g., at 10–12 locations along the Bragg curve. It is suggested that initial studies use multiple human solid tumor cell lines that display different radiation sensitivities and α/β ratios, as well as a limited number of normal cells. Protons (200 MeV) should be used as reference since that is the species used widely clinically. Other ion species of interest are helium, lithium, boron, carbon, oxygen and neon. Two initial beam energies should be used to represent those that might be used clinically, depending on tumor depth in a patient. For practical reasons of targeting a cell monolayer, it is expected that a slightly spread-out Bragg peak, to a width of about 2–3 mm, will be used rather than a pristine Bragg peak. Data could be benchmarked to the previous studies with T-1 human fibroblasts done using helium or neon ions at LBNL, and with HSG human salivary gland tumor cells exposed to carbon ions at the National Institute of Radiological Science [NIRS, Chiba, Japan; summarized in ref. (*6*)].
b. At a more limited number of selected depths along the Bragg curves of each ion in part ''a'', above (e.g., one in entrance plateau, two in SOBP and one just distal to the SOBP), obtain full dose-response curves to allow accurate determinations of RBE and α/β ratio, needed for future treatment planning modeling.

2. Based on the outcome of studies in experiment set 1, with selected tumor cell types, ions and depths in the Bragg curve, conduct in-depth in vitro studies of mode of cell death (mitotic death, apoptosis, permanent arrest, autophagy) to determine whether there are LET-dependent alterations in cell death mechanisms that can be further exploited when using high-LET radiations clinically.
3. Using selected cell types, based on results in experiment set 1, above, obtain full clonogenic survival curves in air and hypoxia with cells irradiated in the SOBP to allow quantification of the oxygen effect.

Recommended Heavy-Ion Biology Investigations That Could Be Undertaken at NSRL with Modest Developments in Physics and Dosimetry

1. Determine RBEs for tumor cure (TCD50) in a selected number of human tumor xenografts or syngeneic rodent tumors grown subcutaneously on a rodent hind limb. Selection of tumors and ion species/energies used could be based on results from *in vitro* studies above.
   a. Such studies could be extended to investigate various dose fractionation patterns allowing for calculations of biologically effective dose (BED) to test fractionation schemes, particularly oligofractionation, and other end points such as immune responses.
   b. For better clinical relevance, studies could be expanded to the use of orthotopic tumors, mentioned above, while requiring greater investments in dose localization and imaging capabilities.
2. Determine the magnitude of the oxygen effect, using TCD50 as the end point, in a selected number of human tumor xenografts grown subcutaneously on a mouse hind limb. Selection of tumors and ion species/energies used could be based on results from studies above.

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